REGULAR ARTICLE

Field performance of STG06L-35-061, a new genetic resource developed from crosses between weed-suppressive indica rice and commercial southern U.S. long-grains

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Abstract

Aims Weed control in rice is challenging, particularly in light of increased resistance to herbicides in weed populations including *Echinochloa crus-galli* (L.) Beauv. Indica rice cultivars can produce high yields and suppress barnyardgrass, but have not been commercially acceptable in the U.S. due to inferior agronomic traits and grain quality. Our objectives were to combine high yield and weed-suppressive characteristics from indica cultivars with commercially acceptable grain quality and plant types from long-grain cultivars grown in the southern U.S.

Methods Crosses between indica and commercial tropical japonica (cv. Katy, and cv. Drew) rice were evaluated for weed suppression and agronomic traits in a breeding program.

Results In some tests, the selection STG06L-35-061 was nearly as weed suppressive as PI 312777, the suppressive parent, and more suppressive than its tropical japonica parents. Its main crop yield is commercially acceptable, and intermediate between PI 312777 and Katy. Its milling quality and cooking quality are similar to long-grain commercial cultivars, and it has resistance to rice blast disease. Marker analyses identified introgressions from the indica parents on chromosomes 1 and 3 of STG06L-35-061 that require further analysis as possible sources of weed suppressive traits.

Conclusions STG06L-35-061 might be suitable for organic rice or reduced input conventional systems.

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Introduction

The detrimental effects of weeds have been a long-standing limitation to the production of rice worldwide. Growing rice cultivars with inherently high levels of weed suppression or weed tolerance might substantially improve the sustainability and affordability of modern rice production in the United States. Potential benefits of such cultivars include reduced herbicide costs, improved control of herbicide-resistant weeds, and compatibility with low input/organic rice systems. Thus, combining traits of commercial importance such as weed suppression potential, grain quality and high yield potential into a product

accepted by the U.S. rice industry is a desirable, but elusive, goal. This paper focuses on a promising selection from breeding efforts at Stuttgart, AR to incorporate weed suppressive traits into high yielding rice cultivars with commercially acceptable grain quality and agronomic traits.

Historically, tropical japonica cultivars with high yield potential and excellent grain quality have been the predominant types grown in the southern U.S. (Moldenhauer et al. 2004). Typically, these cultivars are not inherently weed-suppressive (Gealy et al. 2003; Gealy and Moldenhauer 2012; Gealy and Yan 2012), and are managed with a heavy reliance on herbicides to control weeds (Kendig et al. 2003).

The indica rice subspecies is gaining acceptance in U.S. rice germplasm and breeding programs for its high-yielding, disease-resistant, and potentially weed-suppressive traits (Dilday et al. 2001a, b; Gealy et al. 2005; Gealy and Moldenhauer 2012; Marchetti et al. 1998; Rutger and Bryant 2005; Yan and McClung 2010). Significant suppression of weeds has been demonstrated for a number of indica lines. PI 312777 (T65*2/Taichung Native 1), PI 338046 (IR8*2//B598A4-18-1*2/Taichung Native 1), and Taichung Native 1 (TN 1: a parent of both accessions) have suppressed *Echinochloa crus-galli* (L.) Beauv. (barnyardgrass) and other rice weeds in diverse field and controlled-environment systems worldwide (Dilday et al. 2001a; Gealy et al. 2003; Gealy and Yan 2012; [GRIN] Germplasm Resources Information Network 2012; Kim et al. 2005; Kong et al. 2006, 2008; Seal and Pratley 2010). Potent phytotoxic allelochemicals released by roots of PI 312777 appear to contribute significantly to its weed suppression activity (Chen et al. 2008b; Gu et al. 2009; Kato-Noguchi 2011; Kato-Noguchi and Ino 2005; Kong et al. 2006; Seal and Pratley 2010).

The agronomic and grain quality traits produced by weed suppressive indica lines, however, usually have not met the standards required by the U.S. commercial rice industry. For example, PI 312777, lodges easily and has milling yields <50 gkg^{-1} (head rice) compared to the commercial cultivar, cv. Cypress (61 gkg^{-1}) (Gealy et al. 2003). In some cases, crosses between suppressive indica rice and commercial cultivars have resulted in acceptable grain quality (e.g. RU9701151; Moldenhauer et al. 1999), but these traits were not accompanied by adequate weed suppression activity (Gealy et al. 2005). Recent breeding efforts with

allelopathic rice lines have resulted in improved rice cultivars or germplasm in China and Korea with enhanced weed suppression activity and commercially acceptable quality (Chen et al. 2008b; Kong et al. 2008, 2011; Lee et al. 2008; Ma et al. 2006). Allelopathic rice screening in Cambodia has also revealed promising rice lines for use in their field systems (Pheng et al. 2009a,b).

The objectives of this research were: 1) to identify and field-test crosses between indica (PI 338046 and PI 312777) and tropical japonica, cv. Katy (Moldenhauer et al. 1990) and cv. Drew (Moldenhauer et al. 1998) parents that are adapted to the production systems of the southern U.S., meet U.S. southern long-grain commercial quality standards, and retain high levels of the weed suppression and yield characteristics of the indica lines; and 2) to identify chromosomal introgressions, which originated from the indica lines, that were present in selected weed-suppressive crosses.

Materials and methods

Breeding approach Breeding efforts at the University of Arkansas and USDA-ARS in Stuttgart, AR were undertaken to combine the desirable characteristics of southern U.S. long-grain rice with high-yielding, weed-suppressive indica lines. In earlier work, a selection of F_5 plants from the 1991 cross PI 338046/Katy were bulked to produce STG-94 L-43-130 (RU9701151), which was evaluated in the southern U.S. Uniform Regional Rice Nursery (URRN) in 1997 and observed to have acceptable milling yields (64 gkg^{-1} head rice) (Moldenhauer et al. 1999) and typical southern U.S. long grain cooking quality. In 1997, an F_8 plant of RU9701151 was crossed with a second indica line (PI 312777) with excellent weed-suppressive activity. A selection of F_6 rows from this cross (PI338046/Katy//PI312777) was bulked to produce STG-01 L-30-115. An F_8 plant from this was crossed with the tropical japonica cultivar Drew in 2003 to produce the line STG06L-35-061 (PI338046/Katy//PI312777/3/Drew) which resulted from a bulk of selected F_5 plants. In 2007, this line was evaluated in a preliminary replicated yield test at Stuttgart, AR. The same cross also produced the line STG08L-46-132 (PI338046/Katy//PI312777/3/Drew) from selected bulked F_7 plants. Other selections from

these crossing efforts have produced yields and weed suppression levels intermediate between their indica and tropical japonica parents (Gealy and Moldenhauer 2012; Gealy et al. 2005). One such selection RU0701087 (STG03L-08-047), derived from (PI 338046/Katy//PI 312777) (a separate cross from STG06L-35-061) was also evaluated in the URRN during 2007–2010 due to its previously observed high yield potential.

The initial selections from all crosses were chosen based on agronomic plant type in the F_2, and were advanced through pedigree selection until uniform. Our selection criteria included plant type, milling yield, attributes associated with cooking quality, and rough rice yields. Field tests were conducted for 3 years to identify the lines with the greatest yield potential under weed-free conditions that also possessed commercially acceptable plant and seed quality traits (Moldenhauer et al. 2011). There was no formal screening for weed suppression in the early generations. Plants were selected based on erect stature and height (shorter than ~120 cm). Rice lines satisfying these criteria were grown for seed increase and placed into preliminary weed-infested replicated field trials.

Preliminary yield tests All weed suppression experiments in the field were conducted at the University of Arkansas Rice Research and Extension Center at Stuttgart, AR (34.49° N, 91.55° W) on a DeWitt silt loam (fine smectitic, thermic, Typic Albaqualfs) with 1.2 % organic matter and a pH in water of 5.8. The plot area was managed in a 1-year rice/1-year soybean rotation, and received a broadcast application of 22.4 kg P ha^{-1} as triple superphosphate and 56 kg K ha^{-1} as potassium chloride (muriate of potash) after floating the ground (initial land-levelling) prior to rice or soybean planting each year. Uniform natural populations of barnyardgrass were present in the plots. Low levels of seed of numerous other common rice weed species also were present, but weedy red rice was not present. The preliminary experiments reported in this paper include weed-suppressive germplasm STG06L-35-061, STG08L-46-132, PI 312777, PI 338046, and PI 615031 ('4593'; Dilday et al. 2001b) along with Katy, Drew, cv. Lemont (Bollich et al. 1985), cv. Francis (Moldenhauer et al. 2007), and cv. Bengal (Linscombe et al. 1993) as tropical japonica cultivars, and the commercial hybrid, cv. XL723 (RiceTec, Inc.). Rice was planted on May 21, 2008

and May 19, 2009. Field plots were 2.44 m-long by 1.07 m-wide with six rows spaced 18 cm apart. Rice was drill-seeded approximately 2 cm-deep at a density of 430 seeds m^{-2} (this density was ~3 times that recommended for commercial hybrids). Uniform barnyardgrass infestations were achieved from the combined contributions of the natural soil seed bank and broadcast-seeding of 12 kg live seed ha^{-1} (~1400 live seeds m^{-2}) immediately after rice planting. Plots were firmed with a roller to facilitate seed-soil contact and germination. Propanil (1.12 kg ha^{-1}; one fourth of the recommended rate) was applied pre-flood to all plots so that barnyardgrass would be lightly suppressed (Gealy and Yan 2012), differences between cultivars could be more readily detected, and complete crop loss would not occur if weed populations were unexpectedly high. Bentazon + acifluorfen [Storm], 0.56+ 0.28 kg ha^{-1}, was applied pre-flood in 2008 to control nutsedge (*Cyperus* spp.) and broadleaf weeds. All herbicides were applied using a CO_2-powered backpack sprayer with 8001 flat fan nozzles on 51 cm centers in a shielded boom, calibrated to deliver 94 L ha^{-1} at 159 kPa pressure at a speed of 0.894 m s^{-1}. Urea at 112 kg N ha^{-1} was broadcast over all plots before flood establishment. This sub-optimal rate of fertilization was used to help limit the weed-induced lodging (Gealy et al. 2003) that can result in complete crop loss in these kinds of weed infestation studies. The permanent flood was established on June 19, 2008 and June 23, 2009 and drained on September 10, 2008 and September 18, 2009, respectively. Plots were harvested October 6, 2008 and October 26-November 2, 2009, respectively.

Advanced experiments in weedy and weed-free plots - Weed suppressive candidate lines, STG06L-35-061, RU0701087, and STG08L-46-132 (2010 and 2011 only), three parental cultivars (PI 312777, Katy, and Drew), and Lemont were evaluated under weedy and weed-free conditions across three years at Stuttgart, AR.

Plots that were 3.96 m-long by 1.60 m-wide with nine rows spaced 18 cm apart were drill seeded on May 18, 2009, May 4, 2010, and May 16, 2011. Barnyardgrass was broadcast over the weed-infested plots. Herbicide rates and timings were as follows. In weed-free plots, the initial herbicide treatment (2009, propanil + bensulfuron [Duet], 4.4+0.03 kg ai ha^{-1}, 14 days after crop emergence (DAE); 2010, propanil,

4.4 kg ai ha^{-1}, 5 DAE; 2011, propanil + quinclorac, 3.3+0.28 kg ai ha^{-1}, 9 DAE) was applied when barnyardgrass was at the 2-to 4-leaf stage. A second herbicide treatment (2009, fenoxaprop [Ricestar HT], 0.09 kg ai ha^{-1} + crop oil concentrate [Prime Oil] 1 % v/v, 22 DAE; 2010, propanil + quinclorac, 3.9+ 0.28 kg ai ha^{-1}, 20 DAE; 2011, fenoxaprop [Ricestar HT], 0.11 kg ai ha^{-1}+crop oil concentrate [Prime Oil] 1 %v/v, 31 DAE) was applied before flooding (22 to 27 day after crop emergence). In weedy plots, propanil (1.12, 0.0, and 2.2 kgha^{-1} in 2009, 2010, and 2011, respectively) was applied pre-flood to provide light suppression of the weed pressure anticipated during the growing season.

Urea fertilizer was applied before flood establishment as described above. The permanent flood was established on June 26, 2009, June 14, 2010, and July 1, 2011 and drained on September 18, 2009, September 9, 2010, and September 22, 2011, respectively. Plots were harvested October 17–20, 2009, September 29–30, 2010, and November 2–7, 2011, respectively.

Plant measurements Plant measurements in preliminary yield tests included rice emergence to heading, height and yield at harvest, and visual weed suppression rating (see details below). In addition to these traits, in the advanced experiments, plots were evaluated for rice tillering and weed biomass. In 2009, two 25-cm by 25-cm quadrats outside of the area harvested for yield were subsampled for grass and broadleaf weeds approximately 1 month after flood establishment. In all trials, barnyardgrass was the predominant weed species, but other C$_4$ grass weed species sometimes emerged from the soil (Gealy and Moldenhauer 2012; Gealy and Yan 2012). The number of tillers in a 1-m length from rows 2 and 8 was recorded, and weeds were sampled from the inter-row areas between rows 2 and 3, and rows 7 and 8 in the same locations. The number of tillers per m^2 was determined from a 36 cm×50 cm effective sampling area. In all 3 years, the two weed plant types were separated by hand, dried at approximately 50 °C to a constant mass, and weighed. The total weed biomass was defined as the sum of the biomass of the grass and broadleaf weeds. In 2010 and 2011, these data were recorded at crop maturity (see below).

The percentage of tillers with panicles exerted (heading) in each plot was estimated three times per week throughout the heading period. 'Emergence to heading' was defined as the number of days between the emergence date and the estimated date at which 50 % of panicles had headed. Visual estimates of weed suppression, in which 0 %=no suppression or biomass reduction of barnyardgrass and 100 %=complete suppression or loss of barnyardgrass biomass, were recorded in weedy plots after rice heading.

At crop maturity, ten representative rice plants from the middle 2 m of the interior rows (four in preliminary tests; five in advance experiments) of each plot were measured for height (from the soil surface to the tip of the panicles). All rice plants in this section of the advanced experiments, and the entire plot in preliminary tests were cut and bundled. The bundles were threshed using a stationary Vogle-type thresher. Rough rice grain was weighed and yield was adjusted to 12 % (120 gkg^{-1}) moisture. All data were recorded as described previously (Gealy and Yan 2012). The 'percent reduction' relative to weed-free values, was calculated for emergence to heading, tiller number, height, and yield of rice as previously described (Gealy and Yan 2012).

Multiple-location field trials The cultivars STG06L-35-061, RU0701087, cv. CL142-AR, cv. Cheniere (Linscombe et al. 2006), cv. Cocodrie (Linscombe et al. 2000), and Francis were evaluated as part of the multi-location statewide yield trials conducted by the University of Arkansas rice breeding program (Frizzell et al. 2011). Weed-free plots were evaluated for heading date (days to 50 % heading), height, lodging (on a scale from 1 to 9, where 1=erect and 9=flat), rough rice yield and milling yield (head rice and total rice as gkg^{-1}; determined from two replicates only). Data were obtained from Arkansas Rice Performance Trials (ARPT; three replications) and Stuttgart Initial Trials (SIT; four replications) which were conducted at a total of seven locations from 2009 to 2011. The field locations included Stuttgart, AR, [Dewitt silt loam (soil type)] in 2009 (SIT) and 2010 (ARPT), and the additional ARPT locations at Keiser, AR [Sharkey clay], Carlisle, AR [Dewitt silt loam], Rohwer, AR [Perry clay], and Knobel, AR [sandy loam] in 2010; Corning, AR [Bosket fine sandy loam] in 2011; and Newport, AR [Forrestdale silt loam] and Colt, AR [Calloway silt loam] in 2010 and 2011. The trials were grown in drill-seeded, flood-irrigated systems using cultural recommendations from the Arkansas Cooperative Extension Service for high yield. Typically, rice was seeded in

April or May at 120 kgha^{-1} in nine-row-wide plots (18 cm spacing) of 4.6 m in length. Plots were maintained weed-free by using a tank mixture of 0.30 kgha^{-1} clomazone [Command] plus 0.40 kgha^{-1} quinclorac [Facet] applied pre emergence followed by a tank mixture of 4 kgha^{-1} propanil plus 0.04 kgha^{-1} bensulfuron methyl [Londax] at the four-leaf stage before permanent flood (Frizzell et al. 2011).

Experimental design The experimental design for the 'Preliminary Yield Tests' was a randomized complete block with four replications. The experimental design in the 'Advanced Experiments' was similar to that described previously (Gealy and Yan 2012) and was a split-plot in which main plots were a randomized complete block with four (2009), five (2010), or six (2011) replications. The main plots were rice cultivars, and subplots were weed levels (weedy and weed-free). Data were analyzed using SAS GLIMMIX (SAS Institute Inc., Cary, NC: Ver. 9.2). This mixed model approach, in which years and replications were considered to be random effects, was chosen because it is useful for comparing rice lines when inferences over multiple-environments are of interest (Blouin et al. 2011). Means were separated at the 0.05 level using least squares means with the Tukey-Kramer adjustment. Data analyses for weed biomass and weed suppression values, 'percent rice reduction' values, and analyses for weed-free plots, were conducted separately using a randomized complete block design (Gealy and Yan 2012).

The experimental design for the 'Multi-Location Field Trials' was treated as a randomized complete block in which the cultivar was considered a fixed effect, and replication, year, location, and their interactions were considered random effects (Yan and McClung 2010). There were 2 to 4 replications depending on the location and the variable. Data were analyzed using SAS GLIMMIX. Means were separated as described above.

Genetic marker analysis Healthy, green flag leaves from five separate plants of STG06L-35-061 and Stg-08 L-46-132, and the parental lines, PI 312777, PI 338046, Katy, and Drew were obtained from the plots in the Advanced Experiment on September 23, 2010. The five leaves of each entry were composited and placed in a freezer at −18 C. DNA was extracted from leaves using a rapid extraction method described

by Xin et al. (2003). Eighty-five simple sequence repeat (SSR) markers and one single nucleotide length polymorphism (SNLP) marker (Jia et al. 2004) were selected for this study using information from www.gramene.org and the Cornell University-2001 and IRMI-2003 maps (Temnykh et al. 2001; McCouch et al. 2002). Markers were selected to provide genome-wide coverage of the 12 rice chromosomes in approximately 20 cM intervals. Markers were selected at these intervals to identify the majority of indica introgressions, since Agrama et al. (2007) showed that linkage disequilibrium (LD) in rice is roughly 25–30 cM.

Polymerase chain reaction (PCR) was performed as described in Gealy et al. (2009). Samples were separated on an ABI Prism 3730 capillary DNA sequencer (Applied Biosystems, Foster City, CA 94404) and the sizes of SSR fragments were determined and alleles binned using GeneMapper version 3.7 software (Applied Biosystems, Foster City, CA 94404).

As part of a multi-state URRN test in 2010 STG06L-35-061 was also screened using standard visual, physicochemical, and molecular marker analyses in order to assess its expected cooking quality and resistance to the pathogen (*Magnaporthe oryzae* [formerly *M. grisea*]) causing rice blast disease. RM190 and Exon-6 markers were used to verify amylose content (Chen et al. 2008a and 2010), and "ALK" marker was used to verify gelatinization temperature (Chen et al. 2006). RM224, RM208, and Pi-Indica markers were used to assess the presence of blast resistance genes (*Pi-kh/ks*, *Pi-b*, and *Pi-ta*, respectively) (Fjellstrom et al. 2004; Wang et al. 2010). The development of foliar lesions induced by common races of the blast pathogen was evaluated on greenhouse-grown plants using a visual rating scale (0-to-9; 0=immune, 9=very susceptible).

Results

Preliminary yield tests STG06L-35-061, when evaluated in preliminary yield tests in 2008 and 2009, was statistically similar to its parental cultivars for emergence to heading, height, and yield under weed pressure (Table 1). However, its weed suppression rating was 41 % and 68 % greater than that of Katy and Lemont, respectively. Yields of PI 312777 and XL723 averaged 100 %, 180 %, and 640 % greater than those of Drew, Katy, and Lemont, respectively. In the 2008

Table 1 Weed suppression and agronomic traits in weed infested preliminary field studies conducted at Stuttgart, AR over two years.[a,b,c,d]

Rice cultivar	Emergence to heading days	Height cm	Yield kgha^{-1}	Weed suppression %
STG06L-35-061[c]	83 a	92 ab	3,370 a–c	52 ab
PI 312777	85 a	84 ab	5,540 a	51 ab
PI 338046	87 a	79 bc	3,570 ab	46 a–c
PI 615031	84 a	82 ab	3,770 ab	43 bc
XL723 hybrid[d]	74 c	100 a	5,600 a	54 a
Francis	79 b	83 ab	3,790 ab	45 a–c
Bengal	76 bc	82 ab	4,000 ab	46 a–c
Drew	84 a	80 b	2,780 bc	43 bc
Katy	84 a	84 ab	1,980 bc	37 cd
Lemont	86 a	60 c	749 c	31 d
	$P<0.0001$	$P<0.0001$	$P<0.0001$	$P<0.0001$

[a] Plants were grown in field plots in 2008 and 2009 in a standard drill-seeded, flooded rice production system at Stuttgart, AR. All plots were infested primarily with barnyardgrass, but low levels of other indigenous weed species were sometimes present

[b] Values in table are least-squares means (LSmeans) over 2 years. Values within columns followed by the same letter are not different according to an LSmeans test at $P=0.05$

[c] In 2009 the PI 338046/Katy//PI 312777/3/Drew selection, STG08L-46-132, was also included in the preliminary field test where its emergence to heading was 87 days, height was 78 cm, yield was 2400 kgha^{-1}, and weed suppression was 45 % (as compared to the respective values of 85 days, 92 cm, 3880 kgha^{-1}, and 61 % for STG06L-35-061)

[d] Planting density for the hybrid was the same as that for true-breeding cultivars. This was ~3 times greater than the recommended density for hybrids

preliminary yield test, STG06L-35-061 was observed to suppress barnyardgrass more than other selections in the test, and similar to the levels seen in the PI 312777 allelopathic parental line (data not shown). Based largely on its yield potential and the observation that its weed suppression ratings were similar to those of PI 312777, STG06L-35-061 was included in multi-year advanced field tests.

Advanced experiments in weedy and weed-free plots In advanced tests conducted over three years, STG06L-35-061 yields in weed-free plots were similar to PI 312777, Drew, and RU0701087, and were 15 % and 24 % greater than Lemont and Katy, respectively (Table 2). The height of STG06L-35-061 was similar to that of Drew and Katy, and averaged 17 % greater than that of Lemont, PI 312777, and RU0701087. Emergence to heading for STG06L-35-061 was similar to that of Lemont, Drew, and Katy, and was several days later than for PI 312777. Tillering of STG06L-35-061 was similar to the tropical japonica parents and standards, but was 35 % less than that of PI 312777.

Averaged over weedy and weed-free plots in the advanced three-year test, plant height of STG06L-35-

061 was similar to Drew and Katy, and was 7 % greater than its PI 312777 parent (Table 3). Its yield and weed suppression were intermediate between the high levels of PI 312777 and the low levels of Katy and Lemont, and were similar to those of Drew. The mean values for yield and weed suppression were greater for STG06L-35-061 than for Drew (Table 3), similar to results in the preliminary test (Table 1). The weed densities present in these experiments had minor effects on heading dates (emergence to heading), but reduced rice tiller number, height, and rice yield by at least 40, 12, and 55 %, respectively, for all cultivars except PI 312777 (Table 3). Yield reduction over all cultivars averaged >70 %.

Based on its poor performance in the 2009 preliminary test, STG08L-46-132 was expected to have limited potential as a weed-suppressive line (Table 1 footnote). However, in advanced experiments in 2010 and 2011, yield and weed suppression of STG08L-46-132 were as great as or greater than those of STG06L-35-061 (Tables 3 and 4; Supplemental Table 1), suggesting that both selections may be useful as weed-suppressive genetic resources.

Table 2 Yield and agronomic traits from weed-free advanced field experiments conducted in 2009, 2010, and 2011.[a,b,c,d]

Rice cultivar	Year	Emergence to heading days	Tillers no. m^{-2}	Height cm	Yield kgha^{-1}
Drew	2009	87	584	111	6,570
	2010	85	368	123	4,620
	2011	88	423	116	6,890
	mean	87 a	467 b	117 a	6,050 ab
Katy	2009	88	714	109	4,660
	2010	88	453	124	3,030
	2011	89	485	115	5,620
	mean	89 a	555 b	116 a	4,510 c
Lemont	2009	89	627	87	5,190
	2010	85	463	102	2,880
	2011	90	437	89	6,260
	mean	88 a	516 b	92 d	4,850 c
PI 312777	2009	88	1126	97	7,880
	2010	83	702	110	5,500
	2011	85	597	106	5,790
	mean	85 b	793 a	105 b	6,260 a
RU0701087	2009	81	766	92	5,720
	2010	81	539	104	4,840
	2011	79	497	100	4,970
	mean	80 c	603 b	99 c	5,130 bc
STG06L-35-061	2009	81	697	109	6,560
	2010	86	419	119	4,740
	2011	89	415	115	6,650
	mean	88 a	512 b	115 a	5,600 ab
STG08L-46-132	2009	—	—	—	—
	2010	86	420	118	5,490
	2011	89	412	112	7,710
	mean	88	415	115	6,640
		$P<0.0001$	$P<0.0001$	$P<0.0001$	$P<0.0001$

[a]Values in table are least-squares means (LSmeans). The three-year mean values (over 2009, 2010, and 2011) within columns followed by the same letter are not different according to an LSmeans test at $P=0.05$. Individual year means were included for comparison purposes only and cannot be compared statistically

[b]STG08L-46-132 was excluded from the 2009 experiment and from the statistical analysis

[c]Data for the standards Drew, Katy, Lemont, and PI 312777 in 2009 were obtained from selected 2009 data used in Gealy and Yan (2012)

[d](—) indicates data not available or not applicable

Multiple-location field trials In multi-year, multi-location performance trials conducted under weed-free conditions, heading dates of STG06L-35-061 were in the range of common cultivars–similar to Cocodrie, and 3 to 5 days later than Francis, CL142-AR, and Cheniere (Table 4). Although STG06L-35-061 and CL142-AR were the tallest among the six cultivars compared, lodging potential of STG06L-35-061 was as low as any of the shorter cultivars, suggesting that it has good straw strength. Yield potential of STG06L-35-061 was similar to that of Cheniere, Cocodrie, and CL142-AR, but was 19 % less than that of Francis. Head rice and total milling yields of STG06L-35-061 were commercially acceptable (59.2 and 67.7 %, respectively) and were similar to four commercial cultivars, except that the total milling yield of STG06L-35-061 was 4 % less than that of CL142-AR (Table 4).

Physicochemical measurements as verified by molecular marker analyses obtained from the 2010 URRN, indicate that STG06L-35-061 has desirable cooking and processing quality attributes nearly identical to those of the long-grain cultivars, Francis and CL 142-AR. These include an apparent amylose content of 18.5 % verified by an RM190 marker allele size of 126 bp and Exon-6 allele size of 151 bp; and an alkali spreading value of 3.0 verified by an "ALK" marker allele size of 90 bp (indicative of "intermediate"

Table 3 Means of plant growth and productivity traits of rice and weeds in advanced field experiments conducted over three years.[a,b,c,d,e]

Rice cultivar	Rice								Weeds			Suppression
									Biomass			
	Emergence to heading	Tillers	Height	Yield	Emergence to heading	Tillers	Height	Yield	Grass	Broad leaf	Total	%
	days	no. m^{-2}	cm	kg ha^{-1}	% reduction relative to weed-free				g m^{-2}			
Cultivar main effect (averaged over weed levels)												
Drew	87 ab	362 b	105 ab	3630 a–c	0.38	41 ab	20.6 a	76.0 ab	677 ab	0.25	677 ab	38 bc
Katy	88 a	399 b	104 ab	2660 cd	1.247	53 ab	21.1 a	74.5 ab	658 ab	5.76	662 ab	37 bc
Lemont	88 a	357 b	82 c	2620 d	−0.11	59 a	22.45 a	87.6 a	731 a	6.92	735 a	28 d
PI 312777	85 b	660 a	99 b	4500 a	−0.39	33 b	11.4 b	54.2 c	442 b	0.25	442 b	60 a
RU0701087	80 c	458 b	89 c	2930 b–d	0.946	46 ab	20.4 a	84.6 a	736 a	5.11	740 a	33 cd
STG06L-35-061	87 ab	396 b	106 a	3890 ab	2.2	42 ab	15.3 ab	65.4 bc	554 ab	1.12	554 ab	42 b
STG08L-46-132	86	322	105	4470	3.5	46	18.6	59.7	497	1.7	498	40
	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$	$P = 0.0004$	$P = 0.1206$	$P = 0.0092$	$P = 0.0031$	$P < 0.0001$	$P = 0.0199$	$P = 0.2659$	$P = 0.0183$	$P < 0.0001$
Weed level main effect (averaged over cultivars)												
Without weeds	86 a	568 a	107 a	5470 a	—	—	—	—	—	—	—	—
With weeds	85 b	309 b	87 b	1270 b	—	—	—	—	—	—	—	—
	$P = <0.0032$	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$								

[a] Values in table are least-squares means (LSmeans). The three-year mean values (over 2009, 2010, and 2011) within columns followed by the same letter are not different according to an LSmeans test at $P = 0.05$

[b] STG08L-46-132 was excluded from the 2009 experiment and from the statistical analysis

[c] Data for the standards Drew, Katy, Lemont, and PI 312777 in 2009 were obtained from Gealy and Yan (2012)

[d] The negative numbers for "emergence to heading" values expressed as "% reduction relative to weed-free" indicate that the weed infestation increased the time from rice emergence to heading

[e] (—) indicates data not available or not applicable

Table 4 Agronomic, milling quality, and disease resistance parameters of STG06L-35-061 compared to common commercial cultivars in weed-free, multi-location rice breeding performance trials.[a,b,c]

Rice cultivar	Heading date days to 50 % heading	Height cm	Lodging rating —	Yield kg ha^{-1}	Rice milling yield Head g kg^{-1}	Rice milling yield Total g kg^{-1}	Blast resistance ratings scale of 0 to 9
CL142-AR	83 c	101 a	7.9 a	8430 b	60.5	70.7 a	4.7±3.0
Cheniere	85 bc	85 c	0.3 b	8630 b	61.3	69.6 ab	5.0±2.0
Cocodrie	87 ab	89 bc	1.1 ab	7350 c	57.2	68.5 ab	1.6±2.5
Francis	84 c	93 b	1.4 ab	9640 a	61.4	69.6 ab	5.8±1.1
RU0701087	84 c	90 b	0.4 b	8680 b	62.6	70.2 a	5.7±1.2
STG06L-35-061	88 a	104 a	0.3 b	7780 bc	59.2	67.7 b	1.3±1.5
	$P<0.0001$	$P<0.0001$	$P=0.0101$	$P<0.0001$	$P=0.1684$	$P=0.0012$	——

[a] Plants were grown in field plots using drill-seeded, flooded rice production practices routinely used by the Arkansas Rice Breeding Program (Moldenhauer et al. 2011). Data were obtained from Arkansas Rice Performance Trials or Stuttgart Initial Trials conducted at a total of seven locations in Arkansas from 2009 to 2011

[b] Values in table are least-squares means (LSmeans) over replications, years, and locations. Values within columns followed by the same letter are not different according to an LSmeans test at $P=0.05$

[c] Blast (*Magnaporthe oryzae*) resistance data were obtained from the 2010 URRN greenhouse experiments conducted in Arkansas and Texas. The possible values for ratings ranged from 0 (immune) to 9 (very susceptible). Values presented are means ± standard deviation of resistance ratings recorded for the blast races IB-49, IC-17, IC-1, IG-1, IB54, IE-1, IB-1, and ID-13. Texas data included only races IB-49 and IC-17. Cultivars with ratings ≤3 are considered to be "resistant" or "very resistant". STG06L-35-061 was susceptible to the blast races, IE-1 k, IB 33 and TM2, which were not included in the calculation of the means presented

gelatinization temperature type). These data indicate that, like Francis and CL 142-AR, STG06L-35-061 has conventional U.S. long grain cooking quality (detailed data not shown). Based on grain shape and size, STG06L-35-061 is considered to be a "long-grain" type (data not shown).

URRN marker data also showed that STG06L-35-061 has *Pi-kh* (RM224 allele size=138 bp) and *Pi-ta* (Pi-Indica marker allele size 215 bp) indicating the presence of these key blast resistance genes. In the 2010 URRN greenhouse screening tests in Texas and Arkansas, STG06L-35-061 was resistant or very resistant (rating of 0–3) to a number of blast races, including IB-49, IB-54, IC-1, IC-17, ID-13, IE-1, and IG-1, with an average rating of 1.3±1.5 on a scale of 0 to 9 across these races (Table 4). Its resistance to blast was better than that of CL142-AR and Francis (Table 4), and was generally comparable to that of the indica cv. Rondo, however, it was susceptible to races IE-1 k, IB-33, and TM2 (data not shown).

Genetic marker analysis The genetic composition of STG06L-35-061 in comparison to weed-suppressive indica and non-suppressive tropical japonica parents was based on 86 markers providing genome-wide coverage (Supplemental Table 2). The number of markers per chromosome ranged from 5 on chromosomes 8, 10, and 11, to 11 on chromosome 3. On chromosome 9 there was a large region from about 3-67 cM that is monomorphic between PI 312777, PI 338046, Katy and Drew.

The precise identification of weed-suppression genes or QTL (quantitative trait loci) that are present in STG06L-35-061 cannot be determined directly from our results. However, based on the phenotypic data and the introgressions detected from indica parents, STG06L-35-061 apparently acquired some QTL associated with weed-suppression. We found clear introgressions from the two indica parents in chromosomes 1 (RM490) and 3 (RM3400, RM5626), and introgressions from an unknown source in chromosomes 8 (RM149), 9 (RM219), and 12 (RM247, RM6998) of STG06L-35-061 (Fig. 1; Supplemental Table 2). STG08L-46-132 and STG06L-35-061, which are derived from the same parentage, share the same indica-introgressed alleles on chromosome 3 but differ in indica introgressions on chromosomes 1 and 10 (Supplemental Table 2).
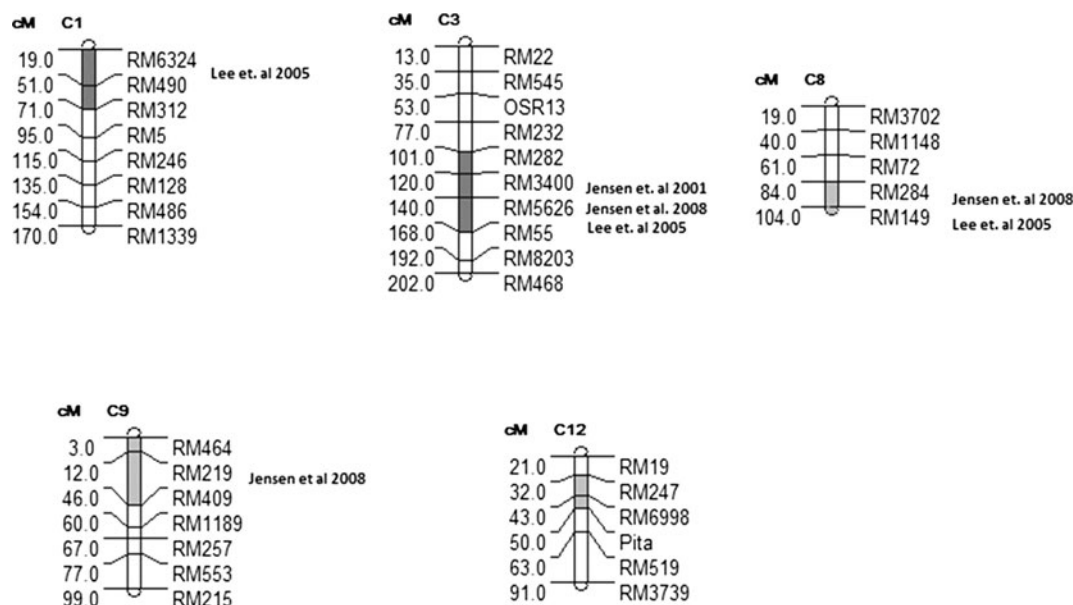
Fig. 1 Maps showing indica introgressions on five chromosomes of STG06L-35-061. Map distances are from the Cornell 2001 map (Temnykh et. al. 2001). Markers not already on that map were positioned based on comparing their physical location to markers already on the Cornell 2001 map. Introgressions are shaded on each chromosome: dark grey indicates an indica introgression from either PI 31277 or PI338046; light grey indicates an introgression from an unknown source. Introgression size represents the maximum possible size. References of allelopathic QTLs that map to a location similar to where an introgression was identified are listed to the side of the introgression. Introgression data were based on 85 SSR markers and one SNLP marker (Jia et al. 2004) providing genome-wide coverage. Supplemental Table 2 provides a list of all markers and the allele sizes detected

Discussion

Field tests Over the past decade, more than 50 selections derived from crosses of weed suppressive germplasm and commercial long grain cultivars have been evaluated as part of the University of Arkansas breeding program (Gealy et al. 2005; Gealy and Moldenhauer 2012). Rice selections with any combination of desirable weed management traits (e.g. weed suppression or yield exceeding that of the non-suppressive parents or standards) were retained for expanded field testing under weedy and weed-free conditions. The vast majority of selections were not evaluated in extended tests due to lack of weed suppression activity. RU9701151 (PI 338046/Katy) produced commercially acceptable yields under weed-free conditions and exhibited weed suppressive activity intermediate between indica and tropical japonica cultivars (Gealy et al. 2005; Gealy and Moldenhauer 2012). However, we discontinued development of this line because its weed suppression potential did not consistently exceed that of tropical japonica cultivars or approach that of the indica cultivars, and it was susceptible to rice blast disease.

Among all of the selections evaluated in our program, STG06L-35-061 exhibited a combination of weed suppression and yield under weedy conditions that was most similar to its weed-suppressive parents. STG06L-35-061 is the first U.S. cultivar derived from allelopathic germplasm that consistently demonstrates weed-suppressive properties against barnyardgrass, a dominant weed species commonly found in U.S. rice production fields. Overall, its yield and weed suppression were intermediate between those of the PI 312777 parent and the tropical japonica parents (Tables 1 and 3). Advantages in weed suppression and yield for indica rice over Katy or Lemont similar to those shown in the present work have been reported previously (Gealy and Moldenhauer 2012; Gealy and Yan 2012). In weed-free, multi-location field tests, the agronomic and grain quality traits of STG06L-35-061 were similar to those of acceptable long-grain commercial varieties grown in the South (Table 4) confirming that this new genetic resource is potentially suited for commercial production.

We observed substantial year-to-year variability in yield and weed suppression due to very diverse climatic

conditions (Supplemental Table 1). The advanced weed suppression experiments were conducted in three consecutive summers in Stuttgart, AR that were notable for unusual weather extremes. For instance, 2009 was a record-setting year for high rainfall, and 2010 and 2011 had extended periods of extremely high temperature (records available online at http://www.ars.usda.gov/Main/docs.htm?docid=21444). These unusual environmental conditions might have impacted performance of STG06L-35-061 and the other cultivars tested. Heavy initial weed populations in advanced experiments in 2011 (199 plants m^{-2}) compared to 2009 and 2010 (86 and 71 plants m^{-2}, respectively; data not shown) probably contributed to the near-complete yield loss (>95 % average) and general lack of weed suppression (31 % average) observed in weedy plots in 2011 (Supplemental Table 1).

Variable results from weed-suppressive rice have been noted in similar studies in Asia. Weed-suppressive effects of different rice cultivars varied with planting method, and cultural management factors such as seeding density, flood depth, and nitrogen fertilization, and the addition of activated charcoal to soil reduced the inhibition of weeds by the allelopathic cultivars, PI312777 and Huagan-1, an improved allelopathic line derived from crosses with PI 312777 (Kong et al. 2008). Although results varied from year-to-year, the allelopathic line, Huagan-3, which was also derived from PI 312777, reduced barnyardgrass biomass by ~80 % compared to rice-free controls (Kong et al. 2011).

Non-allelopathic traits of cultivars such as rapid early plant growth, leaf area, and tiller production tend to enhance suppression of weeds or minimize loss of rice yield from weeds (Gibson et al. 2003; Pérez de Vida et al. 2006). Cultivars with the highest yields often exhibit greater competitiveness against weeds (Gibson et al. 2003; Gealy and Moldenhauer 2012; Gealy and Yan 2012), and generally, that trend was observed in the results of the present studies (Tables 1, 2 and 3). Recent research with high-yielding indica lines in the U.S. suggested that inherently high yielding ability coupled with high tiller production and yield under weed pressure may help improve suppression of and tolerance to weeds in rice under southern U.S. conditions (Gealy and Yan 2012). However, tillering potential of STG06L-35-061 (Table 2) is most consistent with that observed in the commercial tropical japonica types in Gealy and Yan (2012), which was less than in indica rice (e.g. PI 312777) (Gealy and Yan 2012). STG06L-35-061 plant heights were similar to the tallest tropical japonica cultivars and taller than PI 312777, suggesting that this weed-suppressive trait may help compensate for its lack of tillering.

Zhao et al. (2006) used indirect selection based on rice traits measured under weed-free conditions to identify cultivars with competitive abilities in aerobic soils. Traits measured under weed-free and weedy conditions were typically well-correlated, and the traits associated with rapid seedling biomass accumulation were strongly associated with weed suppression and yield with weeds present. They used crop yield, early vigor, and height without weeds to help explain variation in weed suppression under weed competition.

The PI 312777 parent of STG06L-35-061 has been proven to be allelopathic to barnyardgrass. However, incorporating weed suppression and allelopathy with other necessary and desirable traits into commercially useful germplasm is inherently difficult. Khanh et al. (2007) indicated that bottlenecks in the adoption of allelopathy as a weed control tool include quantitative inheritance of the trait; identification of several chemical classes of plant exudates as growth inhibitors; lack of knowledge of the fate and modes of action of the compounds playing major roles in nature; and difficulty in introducing genes responsible for allelopathic activity into target rice cultivars. In a breeding program using allelopathic and nonallelopathic rice varieties in South China, Chen et al. (2008b) showed that allelopathic traits in PI 312777 rice were quantitatively inherited. Crossing between cv. N2S and PI312777 by Chen et al. (2008b) produced high yielding lines which reduced emergence and growth of weeds, and a commercially acceptable allelopathic line, Allelopathic rice No 1. Subsequently, an F_8 line derived from cv. Huahui354×PI312777 with plant phenotype similar to Huahui354 and weed suppression activity similar to PI312777 was released as cv. Huagan-3, the first commercially acceptable allelopathic rice cultivar in China (Chen et al. 2008b). Haugan-3 was grown on an estimated area of >10,000 ha in China from 2009 to 2011 (Kong et al. 2011). Ma et al. (2006) developed an improved cultivar (K21) in Korea by crossing non-allelopathic cv. Donginbyeo (high yielding, good quality) with allelopathic cv. Kouketsumochi and using the single seed descent breeding method. The F_5 generation exhibited desirable agronomic traits from the non-allelopathic parent and allelopathic potential in water extracts, and the F_5 and F_6 generations retained

allelopathic activity and desirable agronomic characteristics under field conditions. Natural products obtained from shoots and roots of K21, an F_6 selection, were confirmed to be allelopathic to barnyardgrass growth in bioassays (Lee et al. 2008; Ma et al. 2006).

In a mass screening, Pheng et al. (2009a) showed that ~4 % of locally adapted Cambodian rice germplasm lines were allelopathic to *Echinochloa colona* in bioassays, and that a smaller subset of these lines were also allelopathic to barnyardgrass and four other weed species. In field evaluations in Cambodia, Pheng et al. (2009b) showed that selected allelopathic rice lines reduced establishment and biomass of barnyardgrass and two other weed species by an average of >70 %, concluding that a combination of resource competition and allelopathic mechanisms were responsible.

Research in Sweden has resulted in notable breeding progress toward improved allelopathic activity with other small grains. Bertholdsson (2005, 2007) used a dual screening approach for germplasm improvement in which seedling bioassays for allelopathic potential and field evaluation of overall weed suppression potential were combined to identify weed-suppressive lines of barley and wheat. Breeding lines of wheat that had exhibited allelopathic activity in agar assays, reduced weed biomass 19 % in the field, whereas a phenotypically similar non-allelopathic breeding parent did not reduce weed biomass (Bertholdsson 2010). Bertholdsson (2011) used factor analysis to show that early season wheat biomass and allelopathic potential were the traits that explained most of the variance in weed biomass, and used partial least squares regression models to predict that an increase in allelopathic activity up to the level of triticale could reduce weed biomass by up to 28 %. Bertholdsson et al. (2012) have further suggested that in vitro selection of wheat-rye substitution lines exhibiting high allelopathic potential against mustard (*Brassica napus*) (roots highly sensitive to rye) could potentially be used to increase weed-suppressive activity in wheat. The breeding selection approach employed in Sweden appears to be a practical and promising means to achieve a viable commercial product for growers because it can exploit key weed-suppressive mechanisms and traits that are acting at the seedling stage as well as throughout the growing season, thus combining to protect the rice crop under field conditions. Worthington and Reberg-Horton (2013) have recently reviewed key breeding issues associated with optimization of allelopathy and competitive ability for weed suppression in cereals.

Genetic marker analysis The introgressions in chromosomes 1, 3, 8, and 9 of STG06L-35-061 map to locations similar to several allelopathic QTLs previously identified for rice (Fig. 1; Bach-Jensen et al. 2001; Jensen et al. 2008; Lee et al. 2005), suggesting that allelopathy might be contributing to weed suppression by STG06L-35-061. Using a population of 142 recombinant inbred lines derived from a cross between cv. IAC 165 and cv. CO 39, Bach-Jensen et al. (2001) found four main QTLs on chromosomes 2, 3, and 8, with the QTLs on 3 an 8 located in the same region of introgression in STG06L-35-061. Using a mapping population derived from a cross between cv. AC1423 (strongly allelopathic indica cultivar) and cv. Aus196 (aus cultivar with low allelopathic potential), 13 QTLs were detected on seven chromosomes for four different allelopathic measurements in a greenhouse study (Jensen et al. 2008). A QTL on chromosome 3 was associated with reduction of barnyardgrass root length and biomass, and shoot length. Lee et al. (2005) found nine QTLs for rice allelopathic effects on barnyardgrass on chromosomes 1, 2, 3, 4, 5, 8, 9 and 12, with the QTLs on chromosomes 1 and 5 being the most allelopathic. The QTL on chromosome 1 is located in the same region as our observed introgression in STG06L-35-061.

Although not clear from the present studies, the non-parental alleles that we detected in STG06L-35-061 (Fig. 1) might have come from our suppressive indica lines. Genetic variability has sometimes been observed in similar germplasm lines obtained from GRIN.

Using a population derived from a cross between PI312777 and cv. Rexmont, Ebana et al. (2001) found seven QTLs for allelopathy on chromosomes 1, 3, 5, 6, 7, 11, and 12, with the QTL on chromosome 6 having the largest effect. Using a double-haploid population derived from cv. ZYQ8/cv. JX17, Zeng et al. (2003), found four QTLs correlated to allelopathy on chromosomes 3, 9, 10 and 12. Lin et al. (2010) and Xiong et al. (2007) tested a mapping population derived from a cross between cv. Dular (allelopathic indica) and Lemont (non-allelopathic japonica). QTLs with additive effects on suppressing the root length of barnyardgrass were mapped to chromosomes 2 and 5. Epistasis analysis revealed three pairs of QTLs in the marker intervals on chromosomes 1, 3, 4, 5 and 10, performing significant additive x additive interactions for allelopathic effects on barnyardgrass root length.

A potential allelopathic component to weed suppression by STG06L-35-061 in the present study was also supported by results from our soil bioassay for allelopathic activity (personal communication, J. Mattice), in which this selection inhibited barnyardgrass seedling growth at levels intermediate between those of PI 312777 and both Katy and Lemont which are non-suppressive (D. Gealy, unpublished data).

Allelopathy considerations Although there is ambiguity in the literature as to the primary compounds responsible for allelopathic activity, and differentiation between allelopathic effects and competition can be difficult (e.g. tillering and root biomass can affect weed suppression), phytotoxins released in root exudates of allelopathic rice include momilactone B, resorcinols, flavone, benzoxazinoids and their respective glycosides, a cyclohexenone, and phenolics (e.g. p-coumaric acid) (Dayan et al. 2005; Kim and Shin 2008; Lin et al. 2010). In-depth work on the potential role of momilactones and other phytoalexins in rice allelopathy has been published. Kong et al. (2004) showed that 3-isopropyl-5-acetoxycyclohexene-2-one-1, momilactone B, and 5,7,4′-trihydroxy-3′,5′-dimethoxyflavone were released from PI 312777 roots at levels sufficient to inhibit growth of barnyardgrass and to be detected in soil. The highest levels of these chemicals were detected in shoots and root exudates suggesting that roots are not an important site for their synthesis or accumulation. However, more recent expression analyses of diterpene cyclase genes responsible for the biosynthesis of diterpene phytoalexins such as momilactones suggest that these compounds are biosynthesized primarily in roots of rice (Toyomasu et al. 2008). Momilactones extracted from hulls of 99 rice varieties inhibited barnyardgrass up to 50 % at concentrations that did not affect rice plants (Chung et al. 2006), and the inhibitory effect increased with concentrations of momilactone A from 250 to 4,000 g a.i./ha. Elevated levels of *p*-coumaric acid in the allelopathic rice cultivar, K21, have been associated with its inhibition of barnyardgrass growth (Ma et al. 2006).

Environmental stress can elicit allelochemical activity and release systems in rice and other plants that can interfere with growth of neighboring plants (Kim and Shin 2008; Dayan et al. 2005). Rice plants have exhibited increased allelopathic potential when in competition with weeds such as barnyardgrass (Wang et al. 2005). Exogenous application of methyl jasmonate and methyl salicylate enhanced the allelopathic activity of IAC165 rice against root growth of barnyardgrass (Bi et al. 2007). Stress from ultraviolet irradiation induced accumulation of momilactones or other phytoalexins in rice leaves (Kodama et al. 1988) and exudation from roots (Kato-Noguchi et al. 2007). Production of momilactones by other species under stress from metals, ultraviolet irradiation, and protein phosphatise inhibitors have also been observed (Kato-Noguchi and Kobayashi 2009). In leaves of Taichung Native 1 and other allelopathic rice cultivars, ultraviolet irradiation has been shown to induce high levels of p-coumaric acid and the enzyme (cinnamic acid 4-hydroxylase) that catalyses the formation of this allelochemical (Shin et al. 2000). Collectively, these reports suggest the possibility that some of the phenomena we occasionally observe in field plots of STG06L-35-061 and PI 312777 might involve environmental-allelopathic interactions. In the 2009 'advanced' experiment (Table 3), mild chlorosis and stunting of barnyardgrass plants was observed at rice heading in plots of STG06L-35-061 (data not shown). A similar unusual phenotypic response also has been observed in barnyardgrass plants in plots of PI 312777 where leaf photosynthesis of this weed was reduced by 30 % relative to its levels in plots of non-suppressive cv. Kaybonnet (Gealy et al. 1998).

PI 312777 has been shown to exhibit high allelopathic potential against weeds under low nitrogen stress conditions, which was accompanied by elevated activation of genes thought to function in the de novo synthesis of allelochemicals (Fang et al. 2010; Song et al. 2008). Such nutrient stress conditions are commonly experienced in organic or low-input agricultural systems. STG06L-35-061 is moderately tall and resistant to lodging (Table 4). These traits should enhance its inherent competitiveness against weeds and its suitability for use in organic or other low input systems. In a review of rice allelopathy and its molecular regulation in responses to biotic and abiotic stress, Lin et al. (2010) reported that under low N stress, increased allelopathic potential was attributable to increased expression level of genes involved in phenolic biosynthesis. Thus, investigation of molecular, ecological rhizospheric biology and systems biology may be helpful to discern the processes and mechanisms involved in rice allelopathy.

Duke et al. (2009) pointed out a recent advance in weed genomics, that all of the key genes in rice

responsible for biosynthesis of momilactone A from its geranylgeranyl pyrophosphate precursor have been isolated and located on chromosome 4 (Shimura et al. 2007). Subsequently, using a reverse-genetics approach, Xu et al. (2012) used knock-outs of relevant diterpene synthase genes to show that momilactones from a japonica rice line contribute to allelopathic suppression of barnyardgrass growth. This work introduced novel genetic evidence for natural product-mediated allelopathy in rice, and provided a potential molecular target for breeding or engineering of rice. Although our genome wide markers occurred on average only every 20 cM, none of our introgressions appeared to be located on chromosome 4. It is possible that we did not have enough marker saturation to identify some introgressions or introgressions on other chromosomes may contain genes involved with regulation of biosynthetic genes for momilactone or other allelochemicals.

Final thoughts Because the inherent yield potential of STG06L-35-061 is not superior to current commercial varieties and hybrids (Tables 2 and 4), it would not likely replace these in conventional production systems in the U.S. However, to our knowledge, STG06L-35-061 is the first improved rice cultivar in the U.S. that was derived from known allelopathic parents and has proved to exhibit weed-suppressive activity and commercially acceptable southern long-grain quality and yield in field tests. Due to the combination of good agronomic traits, grain quality and weed suppression traits of STG06L-35-061, additional crosses with other commercial tropical japonica cultivars and introduced germplasm are underway. The cultivar Francis is of particular interest because of its high yield potential and good weed suppression potential (Gealy and Moldenhauer 2012; Tables 1 and 2).

Natural weed suppression can be affected by biotic and abiotic variables. Thus, STG06L-35-061 and other weed-suppressive cultivars probably will require supplemental weed control inputs or integration with other cultural management tactics except under light weed pressure. Similar sentiments have been reported by others investigating the improvement of allelopathic potential in cereal crops (Bertholdsson 2010, 2011; Kong et al. 2008; Pheng et al. 2009b). Commercially viable yields, good milling and cooking quality, weed suppression potential, blast resistance, and a combination of tall plant height and strong straw (Tables 1, 3 and 4) may position STG06L-35-061 as a potential fit

in low-input or organic rice culture systems, and possibly some conventional systems in which growers are attempting to reduce input costs.

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